Journal of Saudi Chemical Society (2009) **13**, 295–298

King Saud University

Journal of Saudi Chemical Society

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Isolation of a flavonoid from *Feronia limonia*

Javed Intekhab, Mohammad Aslam *

Natural Products Research Division, Post Graduate Department of Chemistry, G.F. College, Rohilkhand University, Shahjahanpur 242001, UP, India

Received 15 April 2009; accepted 14 June 2009
Available online 31 October 2009

KEYWORDS

Feronia limonia;
Rutaceae;
Flavonoid

Abstract The roots and leaves of *Feronia limonia* yielded a flavonoid characterized as 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one along with known imperatorin, bergapten and xanthotoxin. These isolated compounds were characterized by UV, IR, NMR and mass spectral studies.

1. Introduction

Feronia limonia belongs to the family Rutaceae Swingle (syns. *F. elephantum* Correa; *Limonia acidissima* L.; *Schinus limonia* L.) commonly known as wood-apple. It is also called elephant apple, monkey fruit, curd fruit and *katha bel* in India. *F. limonia* has traditionally been used in many herbal remedies such as digestive, stimulant, astringent, carminative and as an antidiarrheal. All the parts of the plants are prescribed in indigenous system of medicine for the treatment of various ailments. Leaves, bark, roots and fruit pulp are all used against snakebite. The bark is chewed with that of *Barringtonia* and applied on venomous wounds. In India the fruit is used as a liver and cardiac tonic, in diarrhea and dysentery (http://www.hort.purdue.edu/newcrop/morton/index.htmlWood-Apple; http://www.worldagroforestry.org/sea/products/AFDbases/AF/index.asp), in effective treatment for hiccough, in sore throat and diseases of the gums. The pulp is poulticed onto bites and stings of venomous insects. Mixture of young leaves juice, milk and candy is given as a remedy for biliousness and intestinal troubles of children (http://www.hort.purdue.edu/newcrop/morton/index.htmlWood-Apple; http://www.worldagroforestry.org/sea/products/AFDbases/AF/index.asp). Fruits, leaves and stem bark of *F. limonia* have been studied for antitumor (Saima et al., 2000; Haque and Chowdhury, 2000), larvicidal (Rahuman et al., 2000) and antimicrobial activity (Rahaman and Gray, 2002; Metha et al., 1983). Fruit pulp showed anti-inflammatory, antipyretic and analgesic activity (Ahamed et al., 2008a). Leaves of the *F. limonia* showed anthelmintic activity (Ahamed et al., 2008b).

The different parts of the plant have been investigated by several workers and found to contain coumarins, furanocoumarins, lignans, alkaloids, steroids and flavonoids. The unripe fruits contain stigmasterol. Root bark yielded osthol, geranyl umbelliferone, marmin, marmesin, aurapten, bergapten, isopimpinellin and fernoil. The heartwood contains ursolic acid and a flavanone glycoside 7-methylporiol-β-D-xylopyranosyl-D-glucopyranoside. The stem bark of *F. limonia* yielded flavanone, alkaloids, coumarins, lignan, sterols and

* Corresponding author. Tel.: +91 9936419611.
E-mail address: Javed786khan_khan@yahoo.com (M. Aslam).

1319-6103 © 2009 King Saud University.
Open access under CC BY-NC-ND license.
doi:10.1016/j.jscs.2009.10.012

Production and hosting by Elsevier

triterpene. Psoralen, bergapten, orientin, vitexin and saponarin have been isolated from leaves (Ahamed et al., 2008b; Shukla and Tiwari, 1971; Agrawal et al., 1989; Talpatra et al., 1973; Banerji et al., 1982; Gupta et al., 1979).

2. Experimental

2.1. General procedures

Ultra violet absorption spectrum was recorded on Perkin–Elmer Lambda Bio 20 UV spectrometer. IR spectroscopy was performed using the KBr disc method on Perkin–Elmer 1710 infrared Fourier transformation spectrometer. NMR spectra were recorded on Bruker AVANCE DRX-300 (300, 100 Hz). Chemical shifts are shown in δ values (ppm.) with tetramethylsilane (TMS) as an internal reference. FEBMS was recorded on JEOLSX 1021/DA-6000 mass spectrometer. Column chromatography was carried using silica gel (60–120 mesh). Chemicals are of analytical-reagent grade were purchased from E-Merck (India).

2.2. Plant material

The roots and leaves of *F. limonia* were collected from the rural areas of the Shahjahanpur District in the month of September 2008. Authentication was achieved by the comparison with the herbarium specimen deposited in the herbarium of the faculty of botany, G.F. College (Rohilkhand University), Shahjahanpur. Fresh or dried plant material can be used as a source for the extraction of secondary plant components. Freshly harvested and dried material is directly used since old dried material stored for a period may undergo some qualitative changes.

2.3. Extraction and isolation

The roots and leaves (1.2 kg) were ground using a grinder, and extracted successively with petrol–ether, dichloromethane and methanol (3 l × 5 times each) using Soxhlet apparatus. From the petrol–ether extract a yellow precipitate (8.7 g), was produced on standing at room temperature. The precipitate was separated from the liquid by filtration and dried off on filter paper. Re-crystallization of a portion (1.7 g) of the precipitate using $CHCl_3$ yielded crystals (60.9 mg) of Fl-1. The rest of the petrol–ether extract was concentrated using a rotary evaporator operating at 50 °C to obtain 6.38 mg of oily substance. A well-stirred suspension of silica gel (100–150 g in petrol–ether 60–80°) was poured into column (150 cm long and 50 mm in diameter). When the absorbent was well settled, the excess of petrol–ether was allowed to pass through column. A portion of this oily material (2.5 g) was digested to well stirred column and eluted with a mobile phase of increasing polarity: petrol–ether/EtOAc/MeOH. Elution with petrol–ether:EtOAc (8:2) afforded a yellow powder (1.23 mg) characterized as Fl-2, whereas on elution with petrol–ether:EtOAc (6:4) afforded Fl-3 (23.2 mg). The isolated three linear furanocoumarins were imperatorin, bergapten and xanthotoxin. The structures of these compounds have been elucidated by UV, IR, MS and comprehensive NMR analysis. Compounds Fl-1 to Fl-3 displayed UV and IR absorption peaks, and ^1H and ^{13}C NMR signals (experimental) characteristic of a coumarin nucleus. The spectroscopic data of compounds Fl-1 to Fl-3 were typical of linear furanocoumarins (Agrawal, 1989; Masuda et al., 1998; Razdan et al., 1987). Coumarins Fl-1 to Fl-3 could be readily identified by comparison of their UV, IR, MS and NMR data with those published for the linear furanocoumarins imperatorin (CS-1), bergapten (FL-2) and xanthotoxin (FL-3), respectively (Agrawal, 1989; Masuda et al., 1998; Razdan et al., 1987).

The MeOH soluble part (52 g) was subjected to a silica gel column chromatography with a gradient of *n*-hexane:EtOAc and then EtOAc:MeOH as eluant. Numerous fractions were collected according to TLC analysis. The fraction of *n*-hexane:EtOAc (7:5) was subjected to a further column chromatography using *n*-hexane:EtOAc (9:1) as the eluant. After recrystallization from MeOH, we obtained 7.5 mg of *Fl-4*.

3. Results and discussion

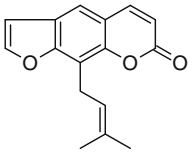
The compound was isolated as yellow amorphous powder from methanol extract by eluting column with *n*-hexane:ethyl acetate (7:5). Compound was first identified as a flavonoid based on its carroty spot on TLC plate visualized with NP-PEG reagent and a positive Shinoda test. This compound was dissolved in H_2SO_4 produced pink color and gave intense red color with Mg/HCl characteristic of flavanones (Mabry et al., 1970). The molecular ion peak at m/z 354 $[M+H]^+$ in its electro spray mass spectrum corresponded to the molecular formula $C_{21}H_{22}O_5$. The *retro* Diels–Alder process from molecular ion was an important decomposition mode found in the mass spectra of flavanone. The ion at m/z 219 was found from the *retro* Diels–Alder fragment-containing ring B and the small peak at 120 was also due to this fragmentation. The IR spectra showed absorption bands at 3410 (–OH), 1655 (α,β-unsaturated carbonyl group), 1613 (aromatic C=C) cm^{-1} functionalities.

According to Mabry et al. (1970), flavanones and isoflavones give similar UV spectra as a result of having little or no conjugation between the A- and B-rings. They are all readily distinguished from flavones and flavonols by their UV spectra, which typically exhibit an intense Band II absorption with only one shoulder or low intensity peak representing Band I. The Band II absorption of isoflavones usually occurs in the region 245–270 nm. Flavanones have a major absorption peak (Band II) in the range 270–295 nm and are therefore clearly distinguished from the spectra of isoflavones (Mabry et al., 1970). The UV absorption spectrum of our compound showed λ_{max} (MeOH) 290, 330 (sh), Band II at 290 nm suggesting the compound to be flavanone (Mabry et al., 1970; Roussis et al., 1987; Mabry and Markham, 1975). Addition of $AlCl_3$ shift reagent caused a bathochromic shift (+22 nm) of Band II confirming the presence of 5-hydroxyl group. The UV spectrum of the compound, confirmed the presence of a hydroxyl function at C-5 in addition to hydroxyl group at C-4′ (Mabry and Markham, 1975).

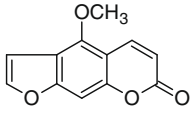
The ^1H NMR spectrum of the compound demonstrated the signals of aromatic, pyron ring and prenyl group. In ^1H NMR the H-2′ and H-6′ pair occur in an identical environment and these are centered at δ 7.32 while the H-3′ and H-5′ pair, also appears as a resonance centered at δ 6.81. Their observed positions in the spectrum are the result of ortho-coupling (J = 8.6 Hz), thus showing a 4′-OH substitution pattern (Mabry and Markham, 1975; Markham, 1982). The ^1H NMR spectrum of the compound revealed a downfield signal

at δ 12.08 assignable to a chelated hydroxyl group at C-5. An ABX system which resonances at δ 5.27 (1H, dd, J = 13.2, 2.7 Hz), δ 3.05 (1H, dd, J = 17.2, 13.2 Hz), and δ 2.75 (1H, dd, J = 17.2, 2.7 Hz) were characteristic of H-2, H-3ax, and H-3eq, respectively, of a flavanone moiety (Mabry and Markham, 1975; Markham, 1982; Ito et al., 1988). The ^{1}H NMR spectrum of the compound displayed signals at δ 6.63 assignable to a phenolic hydroxyl group at C-4′ and a singlet for three protons at δ 3.83 was ascribed to a methoxy group at C-7. The ^{1}H NMR spectrum further displayed a one proton singlet at δ 6.05 was attributed to H-8 proton, it is neither ortho nor meta coupled suggesting that both C-6 and C-7 were blocked. The ^{1}H NMR spectrum also revealed the presence of a γ,γ-dimethyl allyl group with signals at δ 1.66 and δ 1.80 (3H, each, s, 4″, 5″ –CH_3), δ 3.29 (2H, d, J = 6.5 Hz, H-1″) and δ 5.22 (1H, d, J = 6.5 Hz, H-2″) which is correlated to the ^{13}C NMR signals at δ 25.9, 17.5, 21.0 and 122.5, respectively. The attachment of γ,γ-dimethyl allyl group substituent may be at C-6 position (Grayer and Veitch, 2006; Bohalmann et al., 1989). The EI-MS fragmentation of the molecular ion at m/z 354 $[M]^+$ of the compound in its *retro* Diels–Alder fragmentation at ring C yielded diagnostic peaks (experimental) indicating the presence of a hydroxyl and a methoxyl group in ring A, and one hydroxy group in ring B at C-4′ (Harborne and Baxter, 1999), respectively.

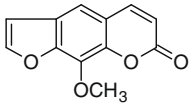
Further the ^{13}C NMR spectrum of the compound showed a total of 19 signals for 21 carbons. A signal was observed at δ 191.2 was allocated to C-4. An additional signal observed at δ 56.4 was ascribed for one methoxy group at C-7. Furthermore two signals were observed resonating at δ 129.2 and δ 113.9 attributed to C-2′/C-6′ and C-3′/C-5′, respectively (Harborne and Baxter, 1999). On the basis of these spectral data the compound was identified as 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one. All these spectral data were in good concurrence with those reported in literature (Harborne and Baxter, 1999; Agrawal, 1989).



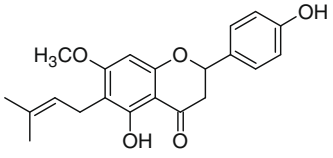
Fl-1



Fl-2



Fl-3



Fl-4

Compound Fl-4: Yellow powder; UV λ_{max}: (MeOH) 290, 330: (MeOH–NaOMe) 325 (+35 nm); (MeOH:$AlCl_3$) 312 (+22 nm); (MeOH–$AlCl_3$–HCl) 308 (+18); (MeOH–NaOAc) 325 (+35 nm); (MeOH–NaOAc–H_3BO_3) 290, 330 nm; IR (KBr) ν_{max}: 3410, 1655, 1613, 1263, 1155 cm^{-1}; Mass spectra m/z: 354 $[M]^+$, 339 $[M-CH_3]^+$, 234 $[M-120]^+$, 311 $[M-CO-CH_3]^+$, 219 $[M-120-CH_3]^+$, 206 $[M-120-CO]^+$, 191 $[M-120-CO-CH_3]^+$, 120; ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 12.08 (1H, br s, 5-OH), 7.32 (2H, d, J = 8.6 Hz, H-2′, H-6′), 6.81 (2H, d, J = 8.6 Hz, H-3′, H-5′), 6.43 (1H, s, 4′-OH), 6.05 (1H, s, H-8), 5.27 (1H, dd, J = 13.0, 3.0 Hz, H-2), 5.22 (1H, d, J = 6.5 Hz, H-2″), 3.83 (3H, s, 7-OCH_3), 3.29 (2H, d, J = 6.5 Hz, H-1″), 3.05 (1H, dd, J = 17.0, 12.8 Hz, H-3), 2.75 (1H, dd, J = 17.0, 3.0 Hz, H-3), 1.80 (3H, s, H-5″), 1.66 (3H, s, H-4″); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C, ppm: 191.2 (C-4), 162.1 (C-7), 161.1 (C-5), 161.0 (C-9), 156.0 (C-4′), 132.2 (C-3″), 130.3 (C-1′), 129.2 (C-2′/6′), 122.8 (C-2″), 113.9 (C-3′/5′), 108.5 (C-6), 102.5 (C-10), 96.3 (C-8), 78.5 (C-2), 56.4 (7-OCH_3), 43.2 (C-3), 26.3 (C-5″), 22.2 (C-1″), 17.5 (C-4″).

4. Conclusion

The isolated three linear furanocoumarins were imperatorin, bergapten and xanthotoxin. The structures of these compounds have been elucidated by UV, IR, MS and NMR spectral analysis. All compounds Fl-1 to Fl-3 displayed UV and IR absorption peaks, and ^{1}H and ^{13}C NMR signals (experimental) characteristic of a coumarin nucleus (Masuda et al., 1998; Razdan et al., 1987; Murray et al., 1982). Compounds Fl-1 to Fl-3 were isolated formerly from *F. limonia* (Shukla and Tiwari, 1971; Agrawal et al., 1989; Talpatra et al., 1973; Banerji et al., 1982; Gupta et al., 1979). However to our knowledge, from the survey of literature 5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-6-(3-methylbut-2-enyl)chroman-4-one was previously unknown from *F. limonia* and hence its first isolation from this natural source.

References

Agrawal, P.K., 1989. Carbon-13 NMR of Flavonoids. Elsevier, Amsterdam.

Agrawal, A., Siddiqui, I.R., Singh, J., 1989. Phytochemistry 28 (4), 1229–1232.

Ahamed, S.M., Swamy, S.K., Jayaverra, K.N., Rao, J.V., Kumar, V.S., 2008a. Pharmacology 3, 852–857.

Ahamed, S.M., Kumar, S.V., Rao, J.V., Jayaveera, K.N., Swamy, S.K., 2008b. Pharmacology 3, 220–223.

Banerji, J., Ghosal, N., Sarkar, S., Kumar, M., 1982. Indian J. Chem. 496.

Bohalmann, F., Zdero, C., Robinson, H., King, R.M., 1989. Phytochemistry 20, 2245–2248.

Grayer, R.J., Veitch, N.C., 2006. In: Andersen, Ø.M., Markham, K.R. (Eds.), Flavonoids: Chemistry, Biochemistry, and Applications. CRC Press Taylor and Francis Group Boca Raton, USA.

Gupta, S.R., Seshadri, T.R., Sharma, C.S., Sharma, N.D., 1979. Planta Med. 36, 95.

Haque, N., Chowdhury, S.A.R., 2000. Fitoterapia 71 (5), 547–552.

Harborne, J.B., Baxter, H., 1999. In: The Handbook of Natural Flavonoids, vols. 1 and 2. John Wiley and Sons, Chichester.

Available from: <http://www.hort.purdue.edu/newcrop/morton/index.htmlWood-Apple> (accessed 10.05.08.).

Available from: <http://www.worldagroforestry.org/sea/products/AFDbases/AF/index.asp> (accessed 10.0508.).

Ito, C., Mizuno, T., Matsuoka, M., Kimura, Y., Sato, K., Kajiura, I., Omura, M., Ju-Ichi, M., Furukawa, H., 1988. Chem. Pharm. Bull. 36, 3292.

Mabry, T.J., Markham, K.R., 1975. In: Harborne, J.B., Mabry, T.J., Mabry, H. (Eds.), The Flavonoids. Chapman and Hall, London.
Mabry, T.J., Markham, K.R., Thomas, M.B., 1970. The Systematic Identification of Flavonoids. Springer-Verlag, New York.
Markham, K.R., 1982. Techniques of Flavonoid Identification. Academic Press, London.
Masuda, T., Takasugi, M., Anetai, M., 1998. Phytochemistry 47, 13.
Metha, P., Chopra, S., Metha, A., 1983. Folia Microbiol. (praha) 28 (6), 467–469.
Murray, R.D.H., Mendez, J., Brown, S.A., 1982. In: The Natural Coumarins: Occurrence, Chemistry and Biochemistry. Wiley, Bristol.
Rahaman, M.M., Gray, A.I., 2002. Phytochemistry 59 (1), 73–77.
Rahuman, A.A., Gopalakrishnan, G., Ghouse, B.S., Arumugam, S., Himalayan, B., 2000. Fitoterepia 71 (5), 553–555.
Razdan, T.K., Qadri, B., Harkar, S., Waight, E.S., 1987. Phytochemistry 26, 2063.
Roussis, V., Ampofo, S.A., Wiemer, D.F., 1987. Phytochemistry 26, 2371.
Saima, Y., Das, A.K., Sarkar, K.K., Sen, A.K., Sur, P., 2000. Int. J. Biol. Macromol. 27 (5), 333–335.
Shukla, J., Tiwari, Shrirama R.D., 1971. Ind. J. Chem. 287.
Talpatra, S.K., Chaudhuri, M.K., Talpatra, B., 1973. Phytochemistry 12, 236.